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Effects of Black Cumin Oil (*Nigella sativa* L.) on Fatty Acid Composition and Cholesterol Level of Eggs in Japanese Quails

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Abstract

The objective of this study was to determine the effects of feeding various levels of *Nigella sativa L.* oil on yolk fatty acid content and egg cholesterol level in Japanese quails. Eighty four female quails at 6 weeks of age were randomly assigned into 4 groups and fed a diet supplemented with 3% hazelnut oil with no *Nigella sativa* oil (Group K, Control), 2% hazelnut oil plus 1% *Nigella sativa* oil (Group L), 1% hazelnut oil plus 2% *Nigella sativa* oil (Group M) or 3% *Nigella sativa* oil with no hazelnut oil (Group N) for 7 weeks. Eggs were collected and weighted daily. Laying performance, feed consumption and egg quality were also evaluated. At the end of the experiment, 3 eggs were obtained for egg yolk fatty acid and cholesterol analysis. Results showed that *Nigella sativa* oil had no significant effects on the feed consumption, egg mass, Haugh unit and bodyweight compared to the control. Egg weight was significantly increased in the groups L, M and N compared to the control. Shell thickness and shell weight of the eggs from the group M were found significantly greater than the control. Adding 2% *Nigella sativa* oil plus 1% hazelnut oil to the diet (Group M) significantly increased the level of saturated fatty acids (SFA) than the other groups ($P < 0.05$). Eggs from the group L had a significantly higher level of C18:1 (n-9) compared to the control ($P < 0.05$). The level of C18:2 (n-6) were significantly lower in eggs from the groups L and N ($P < 0.05$). Cholesterol levels of eggs from the groups were found to be similar.

Keywords: *Nigella sativa Oil; Egg Cholesterol; Yolk Fatty Acid Composition; Japanese Quail*

Introduction

Black cumin (*Nigella sativa* L.) also known as "black seed" grows in Asian and Mediterranean countries. The seed was reported to have many biological properties including anticancerogenic [1,2], anti-parasitic [3,4], anti-diabetic [5,6], diuretic [7,8] and antibacterial activities [9]. Oil extracted from *Nigella sativa* seeds was also shown to have anticarcinogenic [10], antioxidant [11], immunopotentiating [12] hepatoprotective [13,14] and antibacterial [15-17] activities. A recent study reported that treatment with *Nigella sativa* significantly reduced thyroid stimulating hormone and anti-thyroid peroxidase antibodies in patients with Hashimoto's thyroiditis [18].

There are some studies reported on the effects of dietary *Nigella sativa* seed or oil on the performance of poultry. Previously, 2 experiments of a study were conducted in the broiler to show the effects of diets supplemented with essential oil (0.1 or 1 g/kg) or oilseed (10 or 50 g/kg) of *Nigella sativa* seed on body performance [19]. In the first experiment, it was reported that *Nigella sativa* seed and oil affected feed intake and BW positively in the broilers [19]. However, in the second experiment of the same study, no positive results related to those parameters were found [19]. In another study, supplementation of *Nigella sativa* seeds at the levels of 2 or 3% was shown to positively influence egg production, egg weight and shell quality in the laying hens [20]. In the same study, inclusion of *Nigella sativa seeds* was also reported to decrease the level of egg cholesterol [20]. Similarly, Yalçın., *et al.* [21] showed that supplementation of *Nigella sativa* seeds at the levels of 1 or 1.5% had beneficial effects on egg weight, feed conversion ratio (FCR), and egg cholesterol content [21]. Diets supplemented with 1 or 2% *Nigella sativa seeds* significantly improved feed conversion ratio (FCR) in broilers [22].

There have been a limited number of studies associated with the effect of diets supplemented with *Nigella sativa* oil on egg fatty acid composition and yolk cholesterol content in the Japanese quails. Therefore, the objective of this study was to study the effects of *Nigella sativa* oil as an alternative oil source on egg cholesterol and egg fatty acid composition of eggs from Japanese quails fed Nigella sativa oil supplemented diet.

Materials and Methods

This study was conducted in the research farm at the Faculty of Agriculture, in Kahramanmaras Sutcu Imam University, Turkey. In this study, eighty four 6 weeks old laying Japanese quails (*Coturnix coturnix japonica*) were randomly assigned into 4 groups with 3 replicates of 7 birds each (21 birds per group) and fed diets supplemented with 3% hazelnut oil with no additional *Nigella sativa* oil (Group K, Control), 2% hazelnut oil plus 1% *Nigella sativa* oil (Group L), 1% hazelnut oil plus 2% *Nigella sativa* oil (Group M) or 3% *Nigella sativa* oil with no hazelnut oil (Group N) for 7 weeks. Diets were isocaloric and isonutrigenous and contained 2,900 kcal/kg of ME and 20 % CP (Table 1). *Nigella sativa* oil used in this study was purchased from a local company in Urmiya City, Iran. *Nigella sativa* oil was analyzed for fatty acid analysis (Table 2). Water and feed were provided *ad libitum* during the study. The photoperiod was set at 16L: 8D throughout the study. Body weights (BW) of the laying quails were determined at the beginning and end of the study. Feed consumption was calculated on a weekly basis for every replicate in the groups. Eggs were collected and weighed daily. The feed conversion ratio (FCR) was expressed as kilograms of feed consumed per kilogram of egg produced. Eggs were examined for interior and exterior quality. Twelve eggs per group (4 eggs/each replicate) were collected at the end of the study for measuring egg components and parameters. At the end of the experiment, 3 eggs per group were obtained and analyzed for egg cholesterol. Also, three eggs obtained were analyzed for fatty acid composition.

Nutrients	**Diet (%)**
Corn	60.00
[a]Soybean meal (48% CP)	25.85
Calcium carbonate	5.31
[b]DCP	2.06
Lysine	2.22
DL-methionine	0.21
[c]Vegetable oil	3.00
NaCl	0.35
Vitamin and mineral premix	1.00
Calculated analysis	
Dry matters, %	90
Metabolisable energy (kcal/kg)	2900
Crude protein (%)	20
Crude fat (%)	5.53
Crude fiber (%)	1.98
Lysine (%)	0.87
Methionine (%)	0.28
Methionine and Cysteine (%)	0.54
Calcium (%)	23.5
Phosphorus (% total)	7.46

Table 1: *Composition of the basal diet (as dry-matter basis).*

[a]*Soybean meal contained 48% CP.*

[b]*DCP, dicalcium phoshate*

[c]*Vegetable oil was a mixture of Nigella sativa oil and hazelnut oil.*

[2]FAME	%
C4:0	0.08
C6:0	0.01
C14:0	0.01
C16:0	11.57
C16:1 (n-7)	0.70
C17:0	0.10
C17:1	0.16
C18:0	2.65
C18:1 (n-9)	69.88
C18:2 (n-6t)	3.46
C18:2 (n-6c)	9.57
C18:3 (n-3)	0.44
C20:0	0.52
C20:1 (n-9)	0.31
C20:2	0.01
C20:4 (n-6)	0.13
C22:1 (n-9)	0.02
C22:6 (n-3)	0.27
C23:0	0.20
C24:0	0.02
C24:1 (n-9)	0.02

Table 2: *Fatty acid composition of Nigella sativa oil[1].*

[1]Value of the FAMEs are the mean of 2 samples.

[2]Fatty acids methyl esters

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine the effects of *Nigella sativa* oil on egg production, egg weight, feed conversion ratio, egg yolk fatty acid composition and egg cholesterol in laying quails (SPSS Version 21). Significance between individual means was identified using the Duncan multiple range test. Mean differences were considered significant at $P < 0.05$.

Results

The effects of various levels of *Nigella sativa* on the body weight, feed consumption, egg weight, FCR, and egg cholesterol levels (mg/g) were shown in table 3. Supplementing diet with *Nigella sativa* oil had no significant effect on the BW of the laying quails compared to the control group. FCR in the groups K, L, M, and N was similar and 3.12, 3.40, 3.80, and 3.41, respectively. Inclusion of *Nigella sativa* oil in the diets (groups L, M, and N) significantly increased egg weights compared to the control ($P < 0.01$). However, the addition of *Nigella sativa* oil did not influence egg yolk cholesterol content in the groups compared to the control. The level of cholesterol in the eggs from the groups K, L, M and N was 588.78, 592.02, 585.56, and 595.75 mg/g dried yolk, respectively.

Nigella sativa oil at different levels did not have any effect on egg parameters such as yolk weight, albumin weight and Haugh unit (Table 4). In the present study, diet supplemented with 2% *Nigella sativa* oil influenced shell thickness and shell weight significantly ($P < 0.05$). The degree of yolk color increased by adding 2% of *Nigella sativa* oil (group M) into the basal diet ($P < 0.05$). However, a diet including higher levels of *Nigella sativa* seeds (3%) did not have any change in the yolk color.

	[1]Dietary Treatments			
Parameter	**K**	**L**	**M**	**N**
Initial Body Weight (g)	251.9 ± 5.3	253.0 ± 4.3	258.6 ± 5.2	245.0 ± 6.5
Final Body Weight (g)	269.8 ± 6.3	261.9 ± 5.9	265.5 ± 7.0	257.6 ± 9.2
Feed consumption g/bird	29.94 ± 2.10	27.19 ± 2.06	29.78 ± 1.59	28.31 ± 1.65
Feed conversion ratio	3.12 ± 2.54	3.40 ± 0.36	3.80 ± 0.42	3.41 ± 0.36
Egg weight (g)	11.44 ± 0.06^{b}	11.70 ± 0.07^{a}	11.69 ± 0.1^{a}	11.63 ± 0.05^{a}
Egg cholesterol (mg/g)	588.78 ± 5.41	592.02 ± 6.05	585.56 ± 11.43	595.75 ± 9.04

Table 3: *The effects of diets supplemented with Nigella sativa oil on body weight, egg weight, egg production, feed efficiency, and yolk cholesterol content.*

[1]Diets were fed for 7 weeks. Dietary treatments: Group K (Control, 3% hazelnut oil); Group L (2% hazelnut oil plus 1% Nigella sativa oil); Group M (1% hazelnut plus 2% Nigella sativa oil); Group N (0% hazelnut plus 3% Nigella sativa oil).

Values are expressed as means ± SE.

[ab]Mean values in the same row with the same parameter with different letters were significantly different (P < 0.01).

	Dietary Treatments			
Parameters	**K**	**L**	**M**	**N**
Egg weight	10.90 ± 0.17	10.82 ± 0.18	10.63 ± 0.39	10.43 ± 0.41
Yolk Weight (g)	3.75 ± 0.11	3.41 ± 0.10	3.34 ± 0.19	3.45 ± 0.19
Albumin Weight (g)	5.68 ± 0.15	5.80 ± 0.13	5.62 ± 0.23	5.44 ± 0.22
Shell Weight (g)	1.46 ± 0.05^{b}	1.59 ± 0.05^{ab}	1.66 ± 0.04^{a}	1.52 ± 0.07^{ab}
Shell thickness ($mm.10^{-2}$)	0.32 ± 0.03^{b}	0.40 ± 0.03^{ab}	0.41 ± 0.03^{a}	0.34 ± 0.03^{ab}
Yolk Color (Roche fan)	7.66 ± 0.23^{b}	8.33 ± 0.28^{ab}	8.66 ± 0.33^{a}	7.55 ± 0.24^{b}
Haugh Unit	57.52 ± 3.96	56.11 ± 4.51	53.58 ± 7.77	55.70 ± 5.73

Table 4: *The effects of diets[1] supplemented with Nigella sativa oil on egg parameters.*

[1]Diets were fed for 7 weeks. Dietary treatments: Group K (Control, 3% hazelnut oil); Group L (2% hazelnut oil plus 1% Nigella sativa oil); Group M (1% hazelnut plus 2% Nigella sativa oil); Group N (0% hazelnut plus 3% Nigella sativa oil).

[ab]Mean values in the same row with the same parameter with different letters were significantly different (P < 0.01).

The effects of *Nigella sativa* oil on egg yolk fatty acid content were shown on table 5. The level of C14:0 in the eggs from the groups M and N was found to be significantly lower than the control. Inclusion of *Nigella sativa* at the level of 1% *Nigella sativa* L. (group L) significantly decreased the level of C16:0 compared to the control. Eggs from the group M had significantly lower C16:1 (n-7) level compared to the control group. C18:0 is one of the main SFA in the eggs and was found to be significantly lower in the groups L and N compared to the control ($P < 0.05$). The level of C18:2 (n-6) in the eggs from the groups L and N was significantly lower compared to the control ($P < 0.05$). Birds that consumed diet supplemented with 1% *Nigella sativa* L. oil deposited significantly greater levels of C18:1 (n-9), main fatty acid important in the embryonic development, and total MUFA into their eggs compared to the control.

Dietary Treatments				
Fatty acids	**K**	**L**	**M**	**N**
C14:0	0.60 ± 0.01^{a}	0.41 ± 0.01^{ab}	0.40 ± 0.01^{c}	0.47 ± 0.02^{b}
C14:1	0.09 ± 0.0^{ab}	0.08 ± 0.0^{ab}	0.05 ± 0.0^{b}	0.11 ± 0.02^{a}
C15:0	0.02 ± 0.0	0.01 ± 0.0	0.02 ± 0.0	0.02 ± 0.0
C16:0	26.68 ± 0.29^{a}	25.11 ± 0.17^{b}	25.84 ± 0.14^{ab}	26.11 ± 0.60^{ab}
C16:1 (n-7)	5.05 ± 0.07^{ab}	5.15 ± 0.06^{ab}	3.92 ± 0.53^{b}	6.03 ± 0.62^{a}
C17:0	0.06 ± 0.0	0.05 ± 0.0	0.08 ± 0	0.06 ± 0.0

C18:0	5.5 ± 0.04^{b}	5.87 ± 0.43^{b}	8.37 ± 0.82^{a}	5.33 ± 0.34^{b}
C18:1 (n-9)	54.47 ± 0.50^{b}	57.37 ± 0.18^{a}	54.11 ± 0.32^{b}	54.66 ± 1.16^{b}
C18:2 (n-6)	6.01 ± 0.05^{a}	4.40 ± 0.46^{c}	5.73 ± 0.12^{ab}	5.07 ± 0.10^{bc}
C20:1 (n-9)	0.12 ± 0.0^{ab}	0.13 ± 0.0^{a}	0.09 ± 0.01^{c}	0.10 ± 0.0^{c}
C18:3 (n-3)	0.10 ± 0.0	0.08 ± 0.0	0.10 ± 0.0	0.09 ± 0.01
ɣC18:3 (n-6)	0.09 ± 0.0	0.07 ± 0.0	0.08 ± 0.0	0.08 ± 0.0
C20:3 (n-6)	0.06 ± 0.0^{a}	0.03 ± 0.0^{b}	0.03 ± 0.0^{b}	0.02 ± 0.0^{b}
C20:4 (n-6)	0.34 ± 0.0	0.41 ± 0.04	0.37 ± 0.04	0.48 ± 0.03
C22:6 (n-3)	0.02 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0
C24:1	0.05 ± 0.0	0.04 ± 0.0	0.03 ± 0.0	0.04 ± 0.0
SFA	32.87 ± 0.31^{b}	31.46 ± 0.60^{b}	34.72 ± 0.73^{a}	31.99 ± 0.46^{b}
MUFA	59.80 ± 0.42^{bc}	62.78 ± 0.18^{a}	58.22 ± 0.87^{c}	60.95 ± 0.52^{b}
PUFA	6.64 ± 0.04^{a}	5.03 ± 0.50^{b}	6,36 ± 0.08^{a}	5.79 ± 0.10^{ab}
Total	99.31 ± 0.12	99.27 ± 0.80	99.31 ± 0.05	98.75 ± 0.62

Table 5: *The effects of diets supplemented with Nigella sativa oil on the fatty acid composition of the egg yolks.*

^{abc}Means with different superscripts within the same row are significantly different (P < 0.05).

ΣSFA = Total saturated fatty acids; ΣMUFA = Total monounsaturated fatty acids; ΣPUFA = Total polyunsaturated fatty acids.

Discussion

Data regarding the effects of supplementing diet with *Nigella sativa* seed on the BW of chickens and FCR are controversial. A study showed that dietary *Nigella sativa* seed at the level of 1 or 3% significantly ($P < 0.01$) increased final BW in laying hens [23]. However, research by others showed that supplementation of *Nigella sativa L.* seeds in diets of chickens did not have any effect on the BW [20,21]. In contrast to the studies above [20,21,23], it was shown that addition of *Nigella sativa* seeds into the diet significantly decreased BW of the chickens [24]. Inclusion of *Nigella sativa* in the diets was reported to improve FCR the laying hens [21]. In a study conducted in chickens, the best FCR was obtained when diet was supplemented with 2% *Nigella sativa* [17]. However, Aydin., *et al.* [20] showed that the inclusion of *Nigella sativa* in the layer diet did not improve FCR of the hens. Similar to the study by Aydin., *et al.* [20], another study conducted in Japanese quails showed that inclusion of *Nigella sativa* oil had no effect on the body weight gain and FCR [25]. Also feeding laying chickens with diets containing *Nigella sativa* oil did not have any effect on FCR and feed consumption [26]. In the present study, supplementation of *Nigella sativa* oil did not influence final BW, feed consumption and FCR in the laying quails. Inclusion of *Nigella sativa* seeds at the level of1.5% in the diet was shown to raise hen-day egg production from 59 to 77% [27]. It was also shown that supplementation of *Nigella sativa* seeds at the level of 0.5, 1, or 1.5% significantly increased egg weights [21,27,28]. The feeding of powdered *Nigella sativa* seeds to laying hens was shown to lower serum cholesterol and triacylglycerol concentrations, which indeed was associated with a decrease in egg yolk cholesterol contents [27]. Also, El-Bagir., *et al.* [23] reported that feeding diets supplemented with 1 and 3% *Nigella sativa* seeds for a period of three months reduced egg yolk total cholesterol by 34 and 42%. Aydin., *et al.* [20] showed that diets supplemented with 2 or 3% *Nigella sativa* seeds significantly ($P < 0.05$) decreased the concentration of egg yolk cholesterol. In a study conducted in laying hens, diets supplemented with different level of *Nigella sativa* seed powder significantly decreased the levels of serum triglycerides and egg yolk cholesterol of laying hens [29]. *Nigella sativa* seeds (1 and 1.5%) supplementation in layer diets significantly (P < 0.05%) reduced serum and yolk total cholesterol, LDL-cholesterol, triglycerides content and increased HDL-cholesterol [28]. Aydin., *et al.* [20] reported that dietary *Nigella sativa* at the level of 3% was effective to increase the egg weight and egg production significantly ($P < 0.05$) compared to the control. A study conducted in the Japanese quails showed that 0.1% *Nigella sativa* extracts in the diets improved egg production and egg weight significantly (P < 0.05) [25]. Nasir., *et al.* [28] demonstrated that supplementation *of Nigella sativa* seeds at level 0.5, 1 and 1.5 % in layer diets improved egg production and shell thickness. In contrast to the studies above, El-Bagir., *et al.* [23] reported that supplementation of 1 or 3% *Nigella sativa* seed to diet decreased egg production by approximately 9 or 16%, respectively. However, the

results of the present study showed that supplementation of the diet with *Nigella sativa* oil had no effect on egg production compared to the control. Similar to the present study, inclusion of *Nigella sativa* oil in the laying hens' diet at the levels of 1, 2 or 3 ml per kg did not influence egg production and egg weight [26]. However, in the present study, inclusion of the oil significantly increased the egg weights of the quails ($P < 0.05$).

Research showed that inclusion of *Nigella sativa* seeds into the laying hens' diet significantly decreased egg cholesterol. Eggs are excellent source of amino acids, fatty acids, vitamins, and minerals and also contain approximately 213 mg of cholesterol [30]. During the past decades, research efforts directed toward decreasing shell egg cholesterol content have centered on genetic selection or alteration of the diet of laying hens with various nutrients, natural products, non-nutritive factors, or pharmacological agents [31]. However, efforts to modify egg cholesterol contents have demonstrated that it is extremely difficult to decrease egg cholesterol by ordinary means. Dietary *Nigella sativa* seed significantly reduced serum cholesterol, LDL-cholesterol, triglycerol content in laying chickens [27-29]. Inclusion of *Nigella sativa* seed also was shown to decrease egg cholesterol [20,21,23,29]. Similarly, to supplementation of *Nigella sativa* seed, inclusion of *Nigella sativa* oil at the levels of 1 ml/kg and 2 ml/kg significantly was reported to decrease egg cholesterol in the laying hens [26]. Recently, dietary supplementation of *Nigella sativa* was shown to decrease serum cholesterol and triglycerides in the laying chickens [17]. However, in contrast to the studies above [20,23,27,29], it was reported that egg cholesterol content was not influenced by dietary *Nigella sativa* seeds [17]. Similarly, the present study showed that inclusion of *Nigella sativa* oil did not have any effect on the egg cholesterol in the Japanese quails.

Dietary *Nigella sativa* seed was reported to be effective to influence egg shell property. It was shown that inclusion of *Nigella sativa* seed at the level of 0.5, 1.0, or 1.5% increased shell thickness and shell weight of the eggs significantly [27,28]. Diet supplemented with 2 or 3% *Nigella sativa* was also reported to increase shell thickness and shell strength (kg/cm^2) of the eggs in the chickens [20]. Recently, Boka., *et al.* [17] showed that inclusion of *Nigella sativa* seeds at 1, 2, and 3% levels significantly increased the shell strength of the eggs in chickens. In contrast to the studies above, Islam., *et al.* [29] reported that seed powder at the levels of 1.5, 3.5 or 4.5% had no effects on physical properties of eggs in chickens. In a study conducted in quails, addition of 0.1% *Nigella sativa* extracts in diets of laying quails was shown to increase yolk and shell weight in the eggs [25]. In the present study, shell weights of the eggs from the groups fed *Nigella sativa* supplemented diets were similar to the control, but shell thickness of the eggs from the group M was found significantly higher than the control (Table 4). Similarly, to the present study, the addition of *Nigella sativa* oil to the diet was shown not to have any impact on yolk and albumen weight of eggs [26]. The diet supplemented with 3 ml *Nigella sativa* oil per kg diet was shown to decrease Haugh unit by 6.5% compared to control group [26]. In contrast to this study by Bölükbaşı., *et al.* [26], it was shown that *Nigella sativa* seeds supplementation in layer diets significantly ($P < 0.05$) improved Haugh unit value [17,27,28]. In the present study conducted in Japanese quails, dietary *Nigella sativa* oil did not have any effect on Haugh unit in the eggs (Table 4).

There have been a limited number of studies associated with the effect of diets supplemented with *Nigella sativa* oil on the fatty acid composition egg yolk in the Japanese quails. Inclusion of the *Nigella sativa* in the laying diets significantly influenced fatty acid composition of the eggs in chickens [21]. Diets supplemented with 1% and 1.5% *Nigella sativa* seed was shown to have no effects on the levels of C16:0 and C18:0 compared to the control [21]. However, the seed supplementation was shown to decrease total SFA in the eggs [21]. The present study showed that inclusion of *Nigella sativa* significantly influenced the levels of C16:0 and C18:0 in the eggs of the quails. Eggs from the group L had significantly greater levels of C18:1 (n-9) (Table 5). The level of C18:2 (n-6) in the eggs from the groups fed *Nigella sativa* supplemented diets was significantly lower than the control ($P < 0.05$). Also, in the present study, birds consuming diet supplemented with 1 % *Nigella sativa L.* oil deposited significantly greater levels of C18:1(n-9) and total MUFA into their eggs compared to the control.

Conclusion

In conclusion, *Nigella sativa* oil supplemented diets significantly changed the egg fatty acid composition without affecting feed intake, body weight and physical parameters of the eggs compared to the control. Egg weight significantly increased in the groups L, M, and N compared to the control group. Also, the present study showed that shell thickness and shell weight in the eggs from the group M were found to be greater than the control.

Bibliography

1. Kumara SS and Huat BT. "Extraction, isolation and characterisation of antitumor principle, alpha-hederin, from the seeds of Nigella sativa". *Planta Medica* 67.1 (2001): 29-32.
2. Mbarek LA., *et al*. "Anti-tumor properties of blackseed (Nigella sativa L.) extracts". *Brazilian Journal of Medical and Biological Research* 40.6 (2007): 839-847.
3. Ayaz E., *et al*. "The effect of Nigella sativa oil against Aspiculuris tetraptera and Hymenolepis nana in naturally infected mice". *Saudi Medical Journal* 28.11 (2007): 1654-1657.
4. Mahmoud MR., *et al*. "The effect of Nigella sativa oil against the liver damage induced by Schistosoma mansoni infection in mice". *Journal of Ethnopharmacology* 79.1 (2002): 1-11.
5. Al-Hader A., *et al*. "Hypoglycemic effects of the volatile oil of Nigella sativa seeds". *International Journal of Pharmacognosy* 31.2 (1993): 96-100.
6. Mathur ML., *et al*. "Antidiabetic properties of a spice Plant Nigella sativa". *Journal of Endocrinology and Metabolism* 1.1 (2011): 1-8.
7. Zaoui A., *et al*. "Diuretic and hypotensive effects of Nigella sativa in the spontaneously hypertensive rat". *Therapie* 55.3 (2000): 379-382.
8. Toma CC., *et al*. "Comparative studies on polyphenolic composition, antioxidant and diuretic effects of Nigella sativa L. (Black Cumin) and Nigella damascena L. (Lady-in-a-Mist) seeds". *Molecules* 20.6 (2015): 9560-9574.
9. Arici M., *et al*. "Antibacterial effect of Turkish black cumin (Nigella sativa L.) oils". *Grasas y Aceites* 56.4 (2005): 259-262.
10. Salim EI and Fukushima S. "Chemopreventive potential of volatile oil from black cumin (Nigella sativa L.) seeds against rat colon carcinogenesis". *Nutrition and Cancer* 45.2 (2003): 195-202.
11. Sultan MT., *et al*. "Nigella sativa fixed and essential oil improves antioxidant status through modulation of antioxidant enzymes and immunity". *Pakistan Journal of Pharmaceutical Sciences* 28.2 (2015): 589-595.
12. Swamy SM and Tan BK. "Cytotoxic and immunopotentiating effects of ethanolic extract of Nigella sativa L. seeds". *Journal of Ethnopharmacology* 70.1 (2000): 1-7.
13. Meral I., *et al*. "Effect of Nigella sativa on glucose concentration, lipid peroxidation, antioxidant defence system and liver damage in experimentally induced diabetic rabbits". *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine* 48.10 (2001): 593-599.
14. Mollazadeh H and Hosseinzadeh H. "The protective effect of Nigella sativa against liver injury: a review". *Iranian Journal of Basic Medical Sciences* 17.12 (2014): 958-966.
15. Saxena AP and Vyas KM. "Antimicrobial activity of seeds of some ethnomedicinal plants". *Journal of Economic and Taxonomic Botany* 8 (1986): 291-299.
16. Hanafy MS and Hatem ME. "Studies on the antimicrobial activity of Nigella sativa seed (black cumin)". *Journal of Ethnopharmacology* 34.2-3 (1991): 275-278.
17. Boka J., *et al*. "Effect of different levels of black cumin (Nigella sativa L.) on performance, intestinal Escherichia coli colonization and jejunal morphology in laying hens". *Journal of Animal Physiology and Animal Nutrition* 98.2 (2014): 373-383.
18. Tajmiri S., *et al*. "Nigella Sativa treatment and serum concentrations of thyroid hormones, transforming growth factor β (TGF- β) and interleukin-23 (IL-23) in patients with Hashimoto's Thyroiditis". *European Journal of Integrative Medicine* 8.4 (2016): 576-580.

19. Halle I., *et al*. "Effect of ethereal (essential) oil and oilseed on the growth of broilers". Vitamine und Zusatzstoffe in der Ernaehrung von Mensch und Tier: 7. Symposium Jena-Thuringen, Germany (1999).

20. Aydin R., *et al*. "Black cumin (Nigella sativa L.) supplementation into the diet of the laying hen positively influences egg yield parameters, shell quality, and decreases egg cholesterol". *Poultry Science* 87.12 (2008): 2590-2595.

21. Yalçın S., *et al*. "Effects of dietary black cumin seed (Nigella sativa L.) on performance, egg traits, egg cholesterol content and egg yolk fatty acid composition in laying hens". *Journal of the Science of Food and Agriculture* 89.10 (2009): 1737-1742.

22. Ghasemi H., *et al*. "Effects of black cumin seed (Nigella sativa L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers". *Livestock Science* 164 (2014): 128-134.

23. El Bagir NM., *et al*. "Lipid composition of egg yolk and serum inlaying hens fed diets containing black cumin (Nigella sativa)". *International Journal of Poultry Science* 5.6 (2006): 574-578.

24. El-Sheikh AMA., *et al*. "The effect of feeding different levels of Nigella sativa seeds on layer performance and egg quality characteristics". *Sudan Journal of Veterinary Science and Animal Husbandry* 37 (1998): 121-128.

25. Denli M., *et al*. "Effect of dietary supplementation of herb essential oil on the growth performance, carcass and intestinal characteristics of quail (Coturnix coturnix japonica)". *South African Journal of Animal Science* 34.3 (2004): 174-179.

26. Bölükbaşi ŞC., *et al*. "Effect of feeding Nigella sativa oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and Escherichia coli count in feces". *Archiv fur Geflugelkunde* 73.3 (2009): 167-172.

27. Akhtar MS., *et al*. "Effect of feeding powdered Nigella sativa L. seeds on poultry egg production and suitability for human consumption". *Veterinarski Arhiv* 73.3 (2003): 181-190.

28. Nasir Z., *et al*. "Effect of kalongi (Nigella sativa) seeds on egg production and quality in white Leghorn layers". *Journal of Animal and Plant Sciences* 15.1-2 (2005): 22-24.

29. Islam MT., *et al*. "Nigella sativa L. supplemented diet decreases egg cholesterol content and suppresses harmful intestinal bacteria in laying hens". *Journal of Animal and Feed Sciences* 20 (2011): 587-598.

30. USDA. "Nutrient Content of Foods. Dairy and Egg Products Handbook 8-1". USDA, Washington, DC (1991).

31. Elkin RG. "Reducing shell egg cholesterol content. II. Review of approaches utilizing non-nutritive dietary factors or pharmacological agents and an examination of emerging strategies". *World's Poultry Science Journal* 63.1 (2007): 5-32.

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